3

The Hematological Parameters and Blood Cell Morphology of the Brown Bullhead Catfish, *Ictalurus nebulosus* (Le Sueur)

(Tables 1-3)

Sheila R. Weinberg,[1] Charles D. Siegel,[2] Ross F. Nigrelli,[3] and Albert S. Gordon[2]

Department of Biology, Graduate School of Arts and Science,
New York University, New York, New York 10003,
and the Osborn Laboratories of Marine Sciences, Brooklyn, New York 11224.

The present research indicates that the various procedures utilized in the study of the hematopoietic systems of the higher classes of the vertebrates are applicable, with some modifications, for the study of the hematology of the poikilotherms. A description is presented of the peripheral blood cell constituents, with an emphasis on the distinction of the various stages of development of the erythrocytic and leucocytic series, reflected by: changes in the nuclear chromatin, location, size and shape of the nucleus, size and shape of the cell, and the state of cytoplasmic basophilia.

One of the aims of this study was to examine some aspects of the erythrocytic system in the normal, bled, and mechanically stressed bullhead catfish. Values were determined for red cell, white cell, reticulocyte, differentials, hematocrit, hemoglobin, and the erythrocyte corpuscular constants. Statistical analyses indicate a significant difference in the red blood cell count, white blood cell count, reticulocyte count, and mean corpuscular volume after bleeding. The evidence supports the view that there is a physiological control, possibly hormonal in nature, responsible for blood cell formation in different species of fish.

Introduction

There is a need for additional information concerning the morphological and physiological characteristics of the blood of different fishes. The suitability of the catfish as an experimental animal in hematology and immunology has been clearly demonstrated in investigations conducted by Dawson (1935) and more recently by Chuba, *et al.* (1968); Wiener, *et al.* (1968); Kuhns, *et al.* (1969); and Baldo and Boettcher (1970).

The purpose of the present investigation was to obtain more quantitative data relating to the hematological parameters and morphology of catfish blood. Careful selection was made of methods previously used and in some cases a few modifications were instituted to improve upon specific procedures.

[1] Pre-doctoral Trainee, National Institutes of Health Training Grant 2-T01-HE05645-06, National Heart and Lung Institute, National Institutes of Health, United States Public Health Service.

[2] Supported by research grant 5-R01-HE03357-14, National Heart and Lung Institute, National Institutes of Health, United States Public Health Service.

[3] Supported by Scaife Family Charitable Trusts, a grant to the New York Zoological Society.

The blood cell values obtained from these fish may reliably be considered as representing the normal blood picture of the brown bullhead with the question as to a possible difference from wild catfish being unlikely. In this connection, it has been demonstrated that there were no significant differences in the numbers of erythrocytes, hematocrit percentages, and hemoglobin levels between the blood of wild and hatchery-reared lake trout (Piper and Stephens, 1962) and silver salmon fingerlings (Katz and Donaldson, 1950).

Materials and Methods

Aquaria and Fish

A normal group of brown bullhead catfish ranging in size from six to eight inches and weighing less than 50 grams were maintained in fiber glass tubs. A 365-gallon tub was found to be sufficient to support 30 brown bullhead catfish, if it was equipped with a filter system and water pump to maintain a constant flow of water, and two air lines for constant bubbling of air through the closed water system. The temperature, oxygen and pH of the aquaria were measured and recorded daily in the morning.

The temperature was maintained between 22° to 25° C, the oxygen content was above 5 ppm, and the pH was maintained between 6.3 to 8.5. These levels have been recommended by several fish hatcheries (Bureau of Sport Fisheries and Wildlife, 1970; Darragh Company, 1970; and Ralston Purina Company, 1970). The catfish were maintained on a diet of freshly ground beef, and fed daily in the morning. However, the fish were starved for a period of at least 24 hours prior to handling, either for the purpose of mechanically tagging, transfer to an experimental 50 gallon tank, or blood sampling. Even though sex determination of catfish is difficult, an attempt was made following descriptions outlined by some commercial fish journals (American Fish Farmer, Bureau of Sport Fisheries and Wildlife, 1970).

Blood Letting

The unanesthetized bullhead was placed ventral side up, held gently but securely by pressing down the abdomen, just below the pectoral fins. A 0.5 or 1.0 cc tuberculin syringe fitted with a one inch 20-gauge needle, premoistened with a 3.8 percent solution of sodium citrate in Locke's isotonic saline, was oriented with the ventral aorta and inserted gently into the heart, just beneath the pectoral girdle. The syringe plunger was gently pulled back until the desired quantity of blood was obtained. If sufficient blood was not obtained within 30 seconds after the initial penetration, the syringe was withdrawn and the fish was returned to the aquaria. Coagulation of blood, which porbably occurs in the pericardial cavity, could prevent the drawing of the blood sample through the syringe needle (Chuba, *et al.*, 1968; Klontz, 1968; and Dupree, 1970). For repeated blood samplings of fish, blood letting from the heart is recommended (Dupree, 1970). Bleeding from the caudal fin introduces the danger of destroying the caudal vein and bacterial infection. Bleeding was always performed during the same time in the morning to avoid the complication of possible diurnal variations.

It is important to note that 3.8 percent sodium citrate was found to be the only anticoagulant that did not destroy the cell morphology as seen in the blood smear preparations. Following the cardiac blood letting method outlined, fish can be bled from 0.25 ml to 2.0 ml with no mortality.

Determination of Hematological Parameters

Absolute blood cell counts. Erythrocyte counts were made by diluting one part blood with 200 parts of Hayem's solution in a red blood cell diluting pipette, counting the cells in the five smaller squares in a Spencer Bright-line hemocytometer, and multiplying the total count by 10^4 (Smith, *et al.*, 1952; Hesser, 1960; and Klontz, 1968). For the white cell count Shaw's Avian solutions were used (Hesser, 1960; and Klontz, 1968). Both solutions must be filtered prior to use. Blood was drawn up to the 0.5 mark of a red cell diluting pipette; Solution A was added to fill the bulb of the pipette approximately one-half full and mixed. Next, the pipette was filled to the 101 mark with Solution B. A hemocytometer was used and the cells in the four large squares were counted and multiplied by 500. All the counts were made in duplicate for each fish and averaged. The cell counts are representative for each cu mm of whole blood.

Hematocrit determination. The hematocrit was determined by the microhematocrit technique (Larsen and Sneiszko, 1961a). Blood was collected in commercially prepared heparinized capillary tubes and then centrifuged at a high speed for five minutes. Each pair of tubes for each fish was examined to determine the volume of packed red blood cells.

Hemoglobin concentration determination. Several procedures have been used to determine the hemoglobin concentration in catfish blood (Larsen and Snieszko, 1961b; and Larsen, 1964). The cyanmethemoglobin method has been found to be constant and the first choice for the use on catfish blood. A sample of 0.02 ml blood was mixed with 5 ml of Drabkins solution (Wintrobe, 1968). The amount of hemoglobin was then determined spectrophotometrically (at 540 mu) against a commercially prepared standard solution of cyanmethemoglobin (Ortho Diagnostics, Raritan, New Jersey). The hemoglobin is reported as gm/100 ml whole blood relative to man. Using this technique, the assumption was made that fish hemoglobin undergoes the same reactions with potassium-ferricyanide solution as does mammalian hemoglobin (Larsen and Snieszko, 1961b).

Absolute indices. The absolute indices were calculated for the brown bullhead catfish as follows: MCV (Mean Corpuscular Volume) in cubic microns $= \frac{\text{Hematocrit (\%)} \times 10}{\text{RBC } (10^6/\text{cu mm})}$; MCH (Mean Corpuscular Hemoglobin) in picograms $= \frac{\text{Hemoglobin (gm \%)} \times 10}{\text{RBC } (10^6/\text{cu mm})}$; MCHC (Mean Corpuscular Hemoglobin Concentration) in percent Weight/Volume $= \frac{\text{MCH}}{\text{MCV}}$.

Reticulocyte values. A sample of blood was drawn into a plain capillary tube. An equal volume of a one percent Brilliant Cresyl Blue solution in Locke's isotonic saline was drawn into the tube. The mixture was expelled and thoroughly mixed on a piece of parafilm, then taken back into the capillary tube for about five minutes, mixed again and a small drop was then

placed on a methanol cleaned slide to be smeared. After smearing, the slide was air-dried, fixed with absolute methyl alcohol and counterstained with Wright's stain (Humason, 1967). One thousand erythroid cells were counted per slide under oil immersion with the aid of a reticule and reported as per cent of reticulocytes.

Blood cell morphology. A drop of peripheral blood mixed with 3.8 percent sodium citrate was smeared on slides previously cleaned with 50 percent methanol, air-dried and fixed with absolute methanol. The blood smear preparations were then stained with Romanowsky stains: (1) Benzidine stain, Wright's, and Giemsa; (2) Wright's and Giemsa; and (3) May-Grünwald and Giemsa. The smears were examined with an oil immersion objective under 1000 X magnification, and measurements were made with an ocular micrometer.

All procedures were carried out under sterile conditions.

Results

Blood Parameters

In an attempt to determine the normal hematological picture of the brown bullhead, seven fish were mechanically tagged by caudal fin clipping, transferred to a 50-gallon tank, and bled 0.25 ml at two week intervals, thereby allowing a comparison of the blood parameters of individual fish. Similarly, 25 fish were selected at random from a stock normal population, measured, and bled 0.25 ml. The results of the blood analyses and calculations for both groups of fish are listed in Table 1, which shows the total red cell numbers (RBC), total white cell numbers (WBC), reticulocytes (percent per 100 erythroid cells), hematocrit values, hemoglobin concentrations, Mean Corpuscular Volume (MCV, cu μ), Mean Corpuscular Hemoglobin (MCH, picograms, pg), and Mean Corpuscular Hemoglobin Concentration (MCHC, percent). The values are given as the mean, plus or minus one standard error of the mean; the number of fish used in each group is given in parenthesis.

Blood Cell Morphology

Compared to the cells of the erythrocytic series, the identification of the leucocytic series in fish is difficult, especially when attempts are made to discriminate between the granulocyte and agranulocyte. Furthermore, it is even more difficult to distinguish the young granulocytes and agranulocytes from the cells of the thrombocytic group.

The reported descriptions of the agranulocytes in fish are in disagreement, Yuki (1957, 1958) has attempted to resolve the discrepancy in classification of the young and transitional cells in the monocytic and granulocytic groups in rainbow trout.

The difficulty in the task of correctly distinguishing thrombocytes and small lymphocytes lies in the fact (Saunders, 1968b) that some fish species have only mature thrombocytes in their peripheral circulation, while in others both mature and transitional cells can be found.

The following blood cells were detected in the peripheral blood of the brown bullhead: basophilic erythrocytes, reticulocytes, mature erythrocytes, senile or senescent erythrocytes, "nuclear shadows" or basket cells, round lymphocytes, elongated thrombocytes, round thrombocytes, fusiform or spindle-shaped thrombocytes, monocytes, neutrophils, eosinophils, macrophages, and hemocytoblasts. The enucleate erythrocyte or erythroplastid, which arises from the pinching off of a cytoplasmic portion of an erythrocyte, was occasionally found in the peripheral smear preparations but was not considered to be indicative of a blood dysfunction. No basophils were seen in any of the blood smear preparations examined.

The terminology of the cellular components of the brown bullhead used in this paper coincides with those proposed by Jakowska (1956); Chlebeck and Phillips (1969); Yuki (1960, 1963); Srivastava (1968); and Saunders (1967).

The erythrocytic series. The basophilic erythrocyte is slightly oval in shape, containing a centrally located, round nucleus. The size and shape of the basophilic erythrocyte varies, depending on the stage of polychromasia. The cytoplasm assumes a bluish-gray color and the nuclear fine chromatin architecture takes on a light purple color with both benzidine and Romanowsky staining. The cell size measures in the range of $9\mu \times 6\mu$ to $11\mu \times 8\mu$, and the nucleus is 3μ to 4μ in diameter. As with the basophilic erythrocyte, the slightly oval-shaped reticulocyte also varies in size, measuring in the range of $6\mu \times 5\mu$ to $10\mu \times 8\mu$, and its centrally located round nucleus measures 3μ to 4μ in diameter. The fine network of reticulum is clearly seen in a homogeneous pink cytoplasm, if the blood is mixed with a one percent solution of Brilliant Cresyl Blue before staining with Wright's stain.

The circulating normal mature erythrocyte has smooth margins and is predominantly ellipsoidal to oblong in shape and contains a centrally located round nucleus, 2μ to 5μ in diameter. The round erythrocyte measures from 8μ to 10μ in diameter and the ellipsoidal to oblong cell measures $11\mu \times 7\mu$ to $13\mu \times 10\mu$. The homogeneous cytoplasm of this mature cell takes on a pale green color and the nuclear thick chromatin-interchromatin network assumes a dark blue to violet color with benzidine and Romanowsky staining.

Senile or senescent erythrocytes are characterized by a loss of the smooth intact cell membrane and distended cytoplasm, which gives the cell its variability in size and shape. The cell measures 12μ × 10μ with an eccentrically located variably-shaped nucleus, 5μ to 7μ in length. In the most advanced stage of degeneration, the nuclear membrane is no longer intact and its inner contents extend into the distended cytoplasmic portion of the cell. The cytoplasm takes on a pale green color and the nuclear clumped chromatin appears light blue or light purple when stained with benzidine and Wright's stains.

In the disintegrated erythrocytes ("nuclear shadows" or basket cells), the cytoplasm is no longer detected, and the cell therefore assumes a pale pink color when stained with benzidine and Romanowsky stains. An increase in the number of these cells may be due to either mechanical disruption during smear preparation or an increase in the fragility of erythrocytes which may be indicative of the numbers of senescent erythrocytes in the circulating blood. In some of the smear preparations highly refractile red serum granules, 1μ in diameter were found either within the cytoplasm or along the cell membrane of the erythrocyte. Highly refractile vacuoles larger than 1μ in diameter were also observed in cells of the same smear preparations. This infrequent occurrence of cytoplasmic inclusions may have been a result of the smear preparation technique.

Hemocytoblast or Hemoblast. In the circulation, this precursor cell measures from 8μ to 12μ in diameter. The centrally located large nucleus, 7μ to 8μ in diameter, with magenta-stained chromatin filaments and nucleoli, comprises almost the entire volume of the cell. The cytoplasm stains a deep blue with a Romanowsky stain. The hemocytoblast cell is the only direct derivative of the mesenchyme cell and differentiates into the leukogenic and erythrogenic cell series.

Lymphocytes. The round lymphocyte varies in size from 5μ to 7μ in diameter. Its round deeply basophilic nucleus with condensed chromatin comprises the entire volume of the cell. When treated with Romanowsky stains, the deep blue-purple to violet colored nucleus appears to be surrounded by a thin rim of a light pale blue cytoplasm. The irregular cellular outlines of cytoplasmic pseudopods characteristic of these cells are indicative of the lymphocyte's relatively rapid locomotion in the circulation. Vacuoles were not observed in these cells, however, in some instances red-colored azurophilic granules, about 1μ in diameter, approximately 20 per cell, were observed in the cytoplasm.

Thrombocytes. The brown bullhead contains elongated, round, and spindle or fusiform-shaped thrombocytes, some with pointed cytoplasmic terminal processes at one or both ends of the cell. The nucleus is centrally located and varies in outline according to the shape of the entire cell. The dimensions of the cell vary for each type: elongated cell—10μ × 4μ to 14μ × 5μ, nucleus 6μ × 3μ to 9μ × 6μ; round cell—3μ to 4μ in diameter, nucleus 3μ in diameter; spindle or fusiform-shaped cell—5μ × 3μ to 9μ × 3μ, nucleus 4μ × 3μ. The very deep magenta to purple-stained compact nuclear chromatin is characteristic of the round and spindle shaped thrombocyte. In the elongated thrombocyte, the nuclear fine chromatin-interchromatin network takes on a magenta to purple color with Romanowsky stains. The nucleus of the thrombocyte in each of the above mentioned cells is surrounded by a homogeneous very pale blue to colorless cytoplasm, indicating the absence of basophilia. No granules or vacuoles were observed in the cytoplasm in any of the smear preparations, and nuclear indentations of the mature thrombocyte were not a frequent occurrence. Based on the fine network of nuclear chromatin and the slightly deeper blue cytoplasmic color, however, contrary to other investigators (Andrew, 1965), it is our belief that, in the brown bullhead, the elongated cell is the immature thrombocyte.

Granulocytes. The neutrophil is the predominant granulocyte in the circulating blood of the bullhead. It possesses an abundant clear pale blue to colorless cytoplasm surrounding an eccentrically located polymorphic magenta-stained nucleus. The mature neutrophil ranges from 10μ to 17μ in length, with a nucleus measuring from 4μ to 10μ in length. The loosely woven thread-like chromatin pattern of the nucleus may be round, kidney-shaped, ribbon-like, or more or less segmented in shape.

Eosinophils do not appear to be a frequent occurrence in the circulation of all brown bullheads. These large granulocytes, 10μ to 15μ in length, appear to be round to oval in shape. The relatively small, eccentric magenta-stained nucleus with clumped chromatin measures from 4μ to 6μ in length. The characteristic highly refractile eosinophilic red-orange granules, smaller than 1μ in diameter, averaging about 25 per cell, are dispersed throughout the colorless cytoplasm.

Monocytes. Monocytes in the brown bullhead vary in shape and measure 7μ to 14μ in length. The magenta-colored eccentrically-located nucleus is polymorphic in nature, ranging in shape from round, kidney, or bilobed and in size from 4μ to 8μ in length. The abundant cytoplasm of this mature circulating cell takes on a dull gray-blue color with Romanowsky stains. In a few

smear preparations, azurophilic granules, smaller than 1μ in diameter, appear in the cytoplasm. The occurrence of vacuoles in the cytoplasm is also infrequent.

Macrophages. The macrophage in this species of catfish is a very large highly vacuolated cell, frequently containing cellular debris. The appearance of the nucleus depends on the age of the cell. The very old cell contains a small distinct mass of magenta-stained clumped chromatin. In the younger cells, measuring 27μ to 31μ in length, the variably shaped nucleus is 6μ to 10μ in length. The cytoplasm in Romanowsky-stained blood smears takes on a dull gray color.

Discussion

The erythrocyte count, hematocrit, and hemoglobin values of the brown bullhead reported here compare favorably with those reported by Haws and Goodnight (1962), Table 2. Although there were no significant differences in the hematocrit or hemoglobin concentration values, statistical analyses indicate a significant difference in red blood cell count, white blood cell count, reticulocyte count at 17 days after bleeding the seven individual fish ($P \leqq 0.05$). There is also a trend of an increase in the MCV which is to be expected if there is a depletion of the reticulocyte compartment resulting in a red blood cell population consisting predominantly of mature erythrocytes.

The differences observed in the reported values cannot be attributed to any change in the temperature, oxygen level, or pH of the experimental tank, since these values did not alter significantly during the time interval in which the seven tagged fish were housed.

The hematological values obtained for the group of randomly selected 25 fish are given in Table 1. The most striking difference is observed in the total white blood cell values. This difference may be accounted for by the stressed condition to which these fish were subjected when the fish were individually caught for blood sampling. In fact, the differential counts of the individual fish of this group show a definite tendency towards a decrease in lymphocyte count and an increase in thrombocyte count as the fish were subjected to stress for a longer period of time (Table 3). Consequently, the mean differential values for the group of seven fish differ significantly from those for the group of 25 fish. Otherwise, the differential counts of both groups are comparable (Table 3).

Studies with the killifish conducted by Pickford, *et al.* (1971a, 1971b, 1971c) have shown a definite correlation between stress and changes in the abundance of circulating leukocytes, as shown by alternating sequences of leukopenia and leukocytosis. The typical sequence of recovery was described as follows: leukopenia at three min, leukocytosis at 15 min, leukopenia at 30 to 60 min, leukocytosis at 2 hrs, followed by a gradual return to normal.

The only other differential analysis reported for catfish blood has been for the channel catfish species, *Ictalurus punctatus* (Dodgen and Sullivan, 1969). Their findings are comparable with those reported in Table 3, in that the predominant cell found in the peripheral circulation is the lymphocyte.

Differential counts for other species of fish have also reported the lymphocyte as the prevailing white blood cell form, e.g., pike (Mulcahy, 1970), goldfish (Watson and Shechmeister, 1963; Weinreb, 1963), and killifish (Pickford, *et al.* 1971a). This is in contrast to studies done by Saunders with 121 species of

Table 1. The Hematological Parameters of the Brown Bullhead Catfish, *Ictalurus nebulosus* (Le Sueur).

The values are given as the mean, plus or minus one standard error of the mean. The number of fish used in each group is shown in parenthesis.

	RBC 10^6/cu mm	WBC 10^3/cu mm	Retics %	Hct %	Hb gm/100 ml	MCV cu μ	MCH pg	MCHC %
Group I (7)								
T_0	1.79 ±0.067	94.7 ± 3.6	13.0 ± 0.70	24.2 ± 0.59	8.14 ±0.39	136.0 ± 3.60	45.9 ± 3.3	33.1 ± 1.9
T 17 days*	1.47 ±0.116	71.9 ± 6.4	7.13 ± 1.19	23.2 ± 0.76	7.84 ±0.31	160.8 ± 8.11	54.2 ± 2.6	33.1 ± 1.0
T 27 days**	1.44 ±0.056	65.6 ± 8.9	4.92 ± 1.00	24.7 ± 0.93	6.42 ±0.21	172.2 ± 5.88	44.7 ± 1.8	25.7 ± 0.60
Group II (25)	1.69 ±0.051	46.2 ± 2.8	8.79 ± 1.02	30.2 ± 1.00	8.80 ±0.24	180.2 ± 5.18	53.1 ± 1.3	29.0 ± 0.34

* 17 days after first bleeding of 0.25 ml per fish.

** 27 days after first bleeding of 0.25 ml and 10 days after second bleeding of 0.25 ml.

marine fish of Puerto Rico (1966a), 50 species of fish from the Red Sea (1968a), several species of elasmobranchs (1966b), and Gardner and Yevich (1969) with cyprinodontiform species; here the thrombocyte was found to be the predominant cell in differential counts of blood smear preparations.

Summary

A study was made of the hematological parameters and blood cell morphology of a normal population of brown bullhead catfish presented with the intent that these would serve as baseline figures for comparison with values obtained from fish that had been subjected to various forms of hypoxia and other stresses.

The changes in hematological values obtained as a result of blood letting indicated that recovery from an induced state of anemia did not occur before 17 days post bleeding.

Further research is being conducted on the hematological parameters and blood cell morphology of other species of fish, and the physiological control of blood cell formation in different species of fish is presently being examined.

Table 2. Comparison of present results with those of Haws, *et al.* in the brown bullhead catfish.

	Mean	(Range)
Erythrocyte counts: 10^6 cells/cu mm		
Weinberg, *et al.*	1.69	(0.144– 1.74)
Haws, *et al.*	1.22	(0.75 – 1.94)
Hematocrit (%)		
Weinberg, *et al.*	27.2	(23.0 –31.2)
Haws, *et al.*	27.9	(15.0 –47.0)
Hb in gm/100 ml		
Weinberg, *et al.*	8.8	(7.6 –10.0)
Haws, *et al.*	6.9	(4.0 –10.0)
Erythrocyte length (μ)		
Weinberg, *et al.*	–	(9.0 –13.0)
Haws, *et al.*	–	(11.4 –15.9)
Erythrocyte width (μ)		
Weinberg, *et al.*	–	(7.0 –10.0)
Haws, *et al.*	–	(7.6 –11.4)

Literature Cited

American Fish Farmer

1970. Determination of sex of channel catfish. 1:15.

Andrew, W.

1965. Comparative hematology. Grune & Stratton.

Baldo, B. A., and B. Boettcher

1970. ABO(H) erythrocyte specific agglutinins in the serum of the Australian freshwater catfish, *Tandanus tandanus* (Mitchell). Aust. J. Exp. Biol. Med. Sci., 48:241-243.

Bureau of Sport Fisheries and Wildlife

1970. Report to the fish farmers. U.S. Department of the Interior. Feb.

Chlebeck, Anne, and Gary L. Phillips

1969. Hematological study of two buffalo fishes, *Ictiobus cyprinellus* and *I. bubalus* (Catostomidae). J. Fish. Res. Bd. Can., 26: 2881-2886.

Chuba, Joseph, William J. Kuhns, and Ross F. Nigrelli

1968. The use of catfish, *Ictalurus nebulosus* (Le Sueur), as experimental animals for immunization with human secretor saliva and other antigenic materials. J. Immun., 101 (1):1-5.

Darragh Company

1970. Cultured catfish production. Little Rock, Arkansas.

Table 3. Differential Counts of the Brown Bullhead Catfish.

The values are given as the mean, plus or minus one standard error of the mean. The number of fish used in each group is shown in parenthesis.

	Lympho-cytes %	Thrombo-cytes %	Neutro-phils %	Mono-cytes %	Macro-phages %	Hemocy-toblasts %	Eosino-phils %
Group I (7)							
T_0	67.4±4.5	23.6±3.0	6.9±2.5	1.3±0.3	0.08±0.05	0.58±0.30	0.00
T 17 days*	67.9±3.6	22.1±4.7	6.9±3.1	1.7±0.9	0.15±0.10	0.90±0.36	0.00
T 27 days**	66.5±6.2	24.8±6.1	6.1±1.9	1.0±0.3	0.98±0.84	0.45±0.18	0.00
Group II (25)	30.71±2.3	58.6±2.3	7.2±1.1	1.7±0.2	0.82±0.27	0.19±0.05	0.18±0.11

* 17 days after first bleeding of 0.25 ml per fish.

** 27 days after first bleeding of 0.25 ml and 10 days after second bleeding of 0.25 ml.

Dawson, Alden B.
1935. The hemopoietic response in the catfish *Ameirus nebulosus,* to chronic lead poisoning. Biol. Bull., 68 (3):335-346.

Dodgen, Charles L., and Sue Sullivan
1969. Hematological effects of apholate on channel catfish, *Ictalurus punctatus.* Proc. Soc. Exp. Biol. and Med., 131:124-126.

Dupree, Harry D.
1970. Personal communication.

Gardner, George R., and Paul P. Yevich
1969. Studies on the blood morphology of three estuarine Cyprinodonitform fishes. J. Fish. Res. Bd. Can., 26:433-447.

Haws, T. Glenn, and Clarence J. Goodnight
1962. Some aspects of the hematology of two species of catfish in relation to their habitats. Physiol. Zool., 35-36:8-17.

Hesser, E. F.
1960. Methods for routine fish hematology. Prog. Fish. Cult., 22 (4):164-171.

Humason, Gretchen L.
1967. Animal tissue techniques (second edition). W. H. Freeman and Company, San Francisco.

Jakowska, Sophie
1956. Morphologie et nomenclature des cellules du sang des téléostéens. Rev. d'Hematol., 11:519-539.

Katz, Max, and L. R. Donaldson
1950. Comparison of the number of erythrocytes in the blood of hatchery-reared and wild salmon fingerlings. Prog. Fish Cult., 12(1):27-28.

Klontz, George W., and Lynwood S. Smith
1968. Methods of using fish as biological research subjects. *In* Methods of animal experimentation, William Gay, ed. Academic Press, New York, pp. 323-385.

Kuhns, William J., Ross F. Nigrelli, and Joseph V. Chuba
1969. Differences between populations of freshwater catfish defined by blood group antigens and antibodies. J. Immn., 103 (3): 454-459.

Larsen, Howard N.
1964. Comparison of various methods of hemoglobin determination on catfish blood. Prog. Fish Cult., 26 (1):11-15.

Larsen, Howard N., and S. F. Snieszko
1961a. Modification of the microhematocrit technique with trout blood. Trans. Amer. Fish. Soc., 90 (2):139-142.
1961b. Comparison of various methods of determination of hemoglobin in trout blood. Prog. Fish Cult., 23 (1):8-17.

Mulcahy, M. F.
1970. Blood values in the pike, *Esox lucius L.* J. Fish. Biol., 2:203-209.

Pickford, Grace E., Anil K. Srivastava, Anna M. Slicher, and Peter K. T. Pang
1971a. The stress response in the abundance of circulating leucocytes in the killifish *Fundulus heteroclitus.* I. The cold shock sequence and the effects of hypophysectomy. J. of Exp. Zool., 177 (1):89-96.
1971b. II. The role of catecholamines. J. of Exp. Zool., 177 (1):97-108.
1971c. III. The role of the adrenal cortex and a concluding discussion of the leucocyte-stress syndrome. J. of Exp. Zool., 177 (1):109-117.

Piper, Robert G., and Robert F. Stephens
1962. A comparative study of the blood of wild and hatchery-reared lake trout. Prog. Fish Cult., 24 (2):81-84.

Ralston
1970. Catfish book. Ralston Purina Company.

Saunders, Dorothy Chapman
1966a. Differential blood cell counts of 121 species of marine fishes of Puerto Rico. Trans. Amer. Micros. Soc., 85 (3):427-449.
1966b. Elasmobranch blood cells. Copeia, 1966 (2):348-351.
1967. Neutrophils and arneth counts from some Red Sea fishes. Copeia, (3):681-683.
1968a. Differential blood cell counts of 50 species of fishes from the Red Sea. Copeia, (3):491-498.
1968b. Variations in thrombocytes and small lymphocytes found in the circulating blood of marine fishes. Trans. Amer. Micros. Soc., 87 (1):39-43.

Simmons, Arthur
1968. Technical hematology. J. B. Lippincott Company, Philadelphia.

Smith, Charles, William M. Lewis, and Harold M. Kaplan
1952. A comparative morphologic and physiologic study of fish blood. Prog. Fish Cult., 14 (4):169-172.

Srivastava, Anil K.
1968. Studies on the hematology of certain freshwater teleosts. I. Erythrocytes. Anat. Anz. Bd., 123:233-249.

Watson, L. J., I. L. Shechmeister, and L. L. Jackson
1963. The hematology of the goldfish, *Carassius auratus.* Cytologia, 28 (2):118-130.

Weinreb, Eva Lurie
1963. Studies on the fine structure of teleost blood cells. I. Peripheral blood. Anat. Rec., 147 (2):219-238.

Wiener, A. S., J. V. Chuba, E. B. Gordon, and J. W. Kuhns

1968. Hemoglutinins in the catfish (*Ictalurus nebulosus*) injected with saliva from human secretors of various ABO blood groups. Transfusion, 8:226-234.

Wintrobe, Maxwell M.

1967. Clinical hematology. Lea & Febiger, Philadelphia.

Yuki, Ryogo

1957. Blood cell constituents in fish. I. Peroxidase staining of the leucocytes in rainbow trout (*Salmo irideus*). Bull. Fac. of Fish, 8 (1):36-44.

1958. Blood cell constituents in fish. II. Peroxidase staining of leucocytes in the kidney and some other organs. Bull. Fac. of Fish. Hokkaido Univ., 8 (4):264-267.

1960. Blood cell constituents in fish. IV. On the "nuclear shadow" found in blood smear preparations. Bull. Jap. Soc. Sci. Fish, 26 (5):490-495.

1963. A comment on microscopic examination of the blood cell constituents in fish. Bull. Jap. Soc. Sci. Fish, 29 (1):1098-1103.